

Claims

1. An electrode for the electrochemically reversible interconversion of the oxidised and reduced versions of a pyridine nucleotide comprising:
  - an electrically conducting surface;
  - an isolated pyridine nucleotide dehydrogenase module of an enzyme;wherein said isolated pyridine nucleotide dehydrogenase module is applied to the electrically conducting surface.
2. An electrode according to claim 1 wherein said isolated pyridine nucleotide dehydrogenase module of an enzyme is derived from any one of the enzymes selected from the group consisting of: NADH:quinone oxidoreductases (complex I, EC 1.6.5.3 and EC 1.6.99.3); sodium-translocating NADH:quinone oxidoreductases ( $\text{Na}^+$ -NQR); soluble cytoplasmic hydrogenases; and soluble dehydrogenases.
3. An electrode according to claim 2 wherein said isolated pyridine nucleotide dehydrogenase module of an enzyme is the I $\lambda$  subcomplex of bovine mitochondrial NADH:ubiquinone oxidoreductase.
4. An electrode according to any one of the preceding claims wherein said isolated pyridine nucleotide dehydrogenase module of an enzyme is chemically or physically modified such that the pyridine nucleotide dehydrogenase activity is retained.
5. An electrode according to any one of the preceding claims wherein said isolated pyridine nucleotide dehydrogenase module of an enzyme is applied to the surface of the electrode by a method selected from the group consisting of: physisorption;

ionic interaction; chemisorbtion; hydrophobic interaction and binding in a polymer matrix.

6. An electrode according to any one of the preceding claims wherein the dehydrogenase module of an enzyme has been obtained by expression of subunits of said module in a recombinant host cell.

7. An electrode according to any one of the preceding claims wherein said electrically conducting surface is made from a material selected from the group consisting of: carbon; gold; silver; platinum; palladium; tungsten; iridium and well doped semiconductor electrodes such as titanium oxide, indium oxide, tin oxide, or diamond.

8. An electrode according to claim 7 wherein said electrically conducting surface is a carbon material which is chosen from the group consisting of: glassy carbon; highly ordered pyrolytic graphite (HOPG); edge oriented pyrolytic graphite; and graphite.

9. An electrochemical cell comprising:  
a working electrode according to any one of the preceding claims;  
a reference electrode; and  
one or more electrolytes.

10. An electrochemical cell according to claim 9 further comprising an auxiliary electrode.

11. An electrochemical cell according to claim 9 or 10 wherein said reference electrode is a standard reference electrode and is selected from the group consisting of: a

standard calomel electrode; and a silver/silver chloride electrode.

12. An electrochemical cell according to any one of claims 9 to 11 wherein said electrolyte comprises one or more buffers selected from the group consisting of: sodium acetate; potassium phosphate; MES; HEPES; and TAPS.

13. An electrochemical cell according to any one of claims 9 to 12 wherein said electrolyte comprises a body fluid sample.

14. An electrochemical cell according to any one of claims 9 to 13 wherein said electrolyte further comprises:

a substrate;

a second enzyme capable of converting the substrate into a product;

wherein said second enzyme uses a pyridine nucleotide as a cofactor.

15. An electrochemical cell according to claim 14 wherein said second enzyme is a dehydrogenase enzyme.

16. An electrochemical cell according to claim 15 wherein said second enzyme is selected from the group consisting of enzymes with EC numbers: 1.1.1; 1.2.1; 1.3.1; 1.4.1; 1.5.1; 1.7.1; 1.8.1; 1.10.1; 1.11.1.1; 1.11.1.2; 1.12.1; 1.14.12; 1.14.13; 1.16.1; 1.17.1; 1.20.1.

17. A method for effecting the electrochemically reversible interconversion of the oxidised and reduced forms of a pyridine nucleotide in an electrolyte containing the oxidised form and/or the reduced form of the pyridine nucleotide said method comprising applying a potential between the working

electrode and the reference electrode of an electrochemical cell wherein said electrochemical cell is as defined in any one of claims 9 to 16.

18. A method according to claim 17 wherein the potential applied between the working electrode and the reference electrode is such that the current flowing through the electrochemical cell is proportional to the concentration of either the oxidised or the reduced form of the pyridine nucleotide present in the electrolyte.

19. A method according to claim 17 or 18 wherein said potential applied between the working electrode and the reference electrode is an oxidative potential converting the reduced form of the pyridine nucleotide into the oxidised form.

20. A method according to claim 19 further comprising:  
monitoring the current flowing through the electrochemical cell;  
relating said current to the concentration of reduced pyridine nucleotide.

21. A method according to claim 17 or 18 wherein said potential applied between the working electrode and the reference electrode is a reductive potential converting the oxidised form of the pyridine nucleotide into the reduced form.

22. A method according to claim 21 further comprising:  
monitoring the current flowing through the electrochemical cell;  
relating said current to the concentration of oxidised

pyridine nucleotide.

23. A method of altering the relative concentrations of oxidised and reduced forms of a pyridine nucleotide in the electrolyte of an electrochemical cell containing the oxidised and/or reduced forms of said pyridine nucleotide wherein said electrochemical cell is as defined in any one of claims 9 to 16 and wherein said method involves applying a potential difference between the working electrode and the reference electrode of the electrochemical cell.

24. A method of altering the rate of conversion of a substrate to a product in the electrolyte of an electrochemical cell, wherein said electrochemical cell is as defined in any one of claims 14 to 16, comprising applying a potential difference between the working electrode and the reference electrode of the electrochemical cell.

25. A method for measuring the concentration of the substrate in the electrolyte of the electrochemical cell of any one of claims 14 to 16 wherein said method comprises:

applying a potential difference between the working electrode and the reference electrode;

monitoring the current flowing through the electrochemical cell; and

relating said current to the concentration of the substrate.

26. A method according to any one of the preceding claims wherein said pyridine nucleotide is NADH.

27. A method according to any one of the preceding claims wherein said pyridine nucleotide is NADPH.

28. A method according to any one of the preceding claims wherein said pyridine nucleotide is  $\text{NAD}^+$ .

29. A method according to any one of the preceding claims wherein said pyridine nucleotide is  $\text{NADP}^+$ .